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A numerical taxonomic study of Phlebotominae (Diptera: Psychodidae) from China

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Abstract: 【Objective】 Through studying the numerical taxonomy of the species of Phlebotominae from China to explore the phylogenetic position of these species in Phlebotominae. 【Methods】 Cluster analysis was carried out with 40 species of Phlebotominae from China as taxa and 68 morphological characters as index. 【Results】 The result of cluster analysis is consistent with classic taxonomy in the genus classifications of Phlebotominae and subgenus classifications of genus *Phlebotomus*, while there are some differences in the subgenus classifications of genus *Sergentomyia* which is divided into 8 groups by cluster analysis. Two of them are respectively identical with subgenus *Sergentomyia* and *nicnic* group in classic taxonomy. *S. quanzhouensis* and *S. tangi* unite with subgenus *Parrotomyia*. *S. yini* is separated into subgenus *Neophlebotomus*. *S. iyengari*, *S. malayensis*, *S. lanzhouensis*, *S. nankingensis* and *S. turfanensis* aggregate as a group. *S. koloshanensis*, *S. fanglianensis*, *S. yunnanensis* and *nicnic* group unite as a group which has a far phylogenetic relationship with that composed of other species of genus *Sergentomyia*. 【Conclusion】 The result of cluster analysis of Chinese Phlebotominae is consistent with classic taxonomy on the whole. It confirms the reliability of classic taxonomy and also reveals the uncertain classification of some species in genus *Sergentomyia* in classic taxonomy. The difference between the two classifications of genus *Sergentomyia* suggests that refining the taxonomic position of some species and founding a new multispecies genus or subgenus or group at the subgenus level are essential.

Key words: Diptera; Psychodidae; Phlebotominae; sandfly; morphological character; numerical taxonomy; cluster analysis; China

1 Introduction

Phlebotominae belong to Psychodidae in Diptera of Insecta, members of which are blood suckers and characterized by their densely hairy wings and little size. As important medical insects, sandflies can transmit leishmaniasis, bartonellosis (Oroya fever, Carrion's disease), sandfly fever, Toscana virus disease, Chagres virus disease and Punta Toro virus disease. In China, sandflies are the vectors of visceral leishmaniasis and reptile leishmaniasis. The taxonomy of Chinese species of Phlebotominae was initiated by foreign researchers such as Newstead (1916) who defined the first found species in China. Chinese scientists followed the latter work. General census was carried out by Leng (1993), who constructed the classification system (Leng, 1997) according to the stable systematics of Lewis *et al.* (1977) and the research of Old World sandflies of Theodor (1948).

Leng's systematics is used in China at present and regarded as classic taxonomy. There are 47 species reported in China including ungrouped species (*Sergentomyia fanglianensis* and *S. yunnanensis*), some (*S. pooi*, *S. suni* and *S. turfanensis*) needing further study for their indefinite and not widely accepted definition, and two species (*S. tangi and S. lanzhouensis*) which were not classified at the subgenus level. With the exception of the above-mentioned species the Chinese species of Phlebotomiae are divided into five genera, eight subgenera and one group at the subgenus level in classic classifications (Leng, 1997). This study aims at researching the numerical taxonomy of Chinese Phlebotominae for exploring their phylogenetic position of the species in the Phlebotominae.

2 METERIALS AND METHODS

2.1 Selection of taxa

Forty-seven species reported in 5 genera of Chinese

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sandflies were chosen as taxa for cluster analysis. After examination, 7 species (*S. kueichenae*, *S. wuyishanensis*, *S. yaoi*, *S. sumbatica*, *S. wangi*, *S. pooi* and *S. suni*) were rejected for their incomplete description (missing data, only one sex is known). Eventually 40 species were retained. Morphological characters of 25 species were obtained from the sandflies mounted in the slides of our laboratory, which were identified by LENG Yan-Jia. The length characters were got by plotting and measuring with microscope, micrometer and camera lucida. The details of 15 species came from original species reports and other references. The species used and their classic classification positions are listed in Table 1.

Table 1 The species used in this study and their classic classification position

Genus	Subgenus	Species	Number of females	Number of males	Locality	Collecting time*
Chinius		*C. junlianensis* Leng, 1987	10	10	Lingyun, Guangxi	1988
Grassomyia		*G. indica* Theodor, 1958 (Yao and Wu, 1938)	5	5	Zhanjiang, Guangdong	1973
Idiophlebotomus		*I. longiforceps* Wang, Ku & Yuan, 1974 (Wang *et al.*, 1974)	4	8	Congjiang, Guizhou	1982
Phlebotomus	*Adlerius*	*P. chinensis* Newstead, 1916	11	10	Qianshan, Liaoning	1984
		P. fengi Leng & Zhang, 1994	4	2	Longling, Yunnan	1984
		P. longiductus Parrot, 1928 (Perfil'ev, 1968)	2	2	Kuche, Xinjiang	1996
		P. sichuanensis Leng & Yin, 1983	10	10	Lixian, Sichuan	1984
	Anaphlebotomus	*P. stantoni* Newstead, 1914 (Yao and Wu, 1938)	8	10	Zhanjiang, Guangdong	1973
	Euphlebotomus	*P. kiangsuensis* Yao & Wu, 1938	3	3	Lingyun, Guangxi	1988
		P. lengi Zhang, He & Ward, 1994	10	10	Jinggu, Yunnan	1989
		P. tumenensis Wang & Chang, 1963	1	9	Maowen, Sichuan	1977
		P. yunshengensis Leng & Lewis, 1987	6	10	Junlian, Sichuan	1982
	Larroussius	*P. smirnovi* Perfiliew, 1941 (Perfil'ev, 1968)	2	2	Ejinaqi, Inner Mongolia	1990
	Paraphlebotomus	*P. alexandri* Sinton, 1928	3	7	Jiuquan, Gansu	1990
		P. andrejievi Shakirzyanova, 1953 (Chai, 2006)	1	1	Ejinaqi, Inner Mongolia	1990
		P. caucasicus Marzinovsky, 1917 (Chai, 2006)	1	1	Urumchi, Xinjiang	1961
		P. mongolensis Sinton, 1928	8	2	Jiuquan, Gansu	1990
Sergentomyia	*Neophlebotomus*	*S. anhuiensis* Ge & Leng, 1990	7	3	Wanqimen, Anhui	1986
		S. iyengari Sinton, 1933	4	9	Nanfeng, Hainan	1957
		S. khawi Raynal, 1936	10	2	Beijing	1988
		S. koloshanensis Yao & Wu, 1946 (Yao and Wu, 1946)	10	5	Lingyun. Guangxi	1984
		S. malayensis Thedor, 1938	10	10	Nanfeng, Hainan	1986
		S. nankingensis Ho, Tan & Wu, 1954 (He *et al.*, 1954)	3	4	Nanjing, Jiangsu	1952
		S. quanzhouensis Leng & Zhang, 1987	2	7	Quanzhou, Fujian	1983
		S. squamirostris Newstead, 1923	10	10	Wanchu, Anhui	1986
		S. zhengjiani Leng & Yin, 1983	5	5	Lixian, Sichuan	1980
		S. zhongi Wang & Leng, 1991 (Wang and Leng, 1991)	1	6	Songyang, Zhejiang	1986
	Parrotomyia	*S. barraudi* Sinton, 1929	10	10	Wulong, Chongqing	1984
		S. kwangsiensis Yao & Wu, 1941 (Leng and Lin, 1991)	2	1	Debao, Guangxi	1984
		S. rudnicki Lewis, 1978	10	10	Menla, Yunnan	1988
		S. yini Leng & Lin, 1991	4	5	Wulong, Chongqing	1984
		S. sintoni Pringle, 1953 (Chai, 2006)	1	1	Junggar, Xinjiang	1983
		S. sinkiangensis Leng, Lane & Lewis, 1987 (Chai, 2006)	10	10	Turfan, Xinjiang	1958
	nicnic group	*S. bailyi* Sinton, 1931	3	8	Nanfeng, Hainan	1957
		S. campester Sinton, 1931	10	10	Nanfeng, Hainan	1957
	Ungrouped	*S. fanglianensis* Leng, 1964	6	6	Baisha, Hainan	1959
		S. yunnanensis He & Leng, 1991	1	8	Yuanjiang, Yunnan	1989
	Unclassified at	*S. lanzhouensis* Xiong, Jin & Zuo (Chai, 2006)	1	1	Shawan, Xinjiang	1983
	subegenus level	*S. tangi* Xiong, Chai & Jin (Chai, 2006)	1	1	Shawan, Xinjiang	1983
		S. turfanensis Hsiung, Guan & Jin, 1981 (Chai, 2006)	2	1	Turpan, Xinjiang	1974

* All samples were collected between June and October.

2.2 Choice of characters and definition of states

The arguments of choice are availability for all taxa, absence of redundancy, clear definition of the states of characters, the stability of these states at a taxonomic level and comprehensive description of the sandflies. Thus, the result would be objective and close to the description of the natural states. After examination, 68 characters were chosen following Bermúde *et al.* (1991), also with a reference of Leng (1991). All characters are categorical variables for the variability of quantitative characters. Characters and definition of states are listed in Table 2.

Table 2 The characters and their state values for this study

	Character	Character states and their values
1	Shape of wing	oval [0]; acute [1]
2	Length of wing **F**	shorter than 1 500 μm [1]; 1 500 –2 200 μm [2]; 2 201 –2 700 μm [3]; longer than 2 700 μm [4]
3	Length of wing **M**	shorter than 1 500 μm [1]; 1 500 –2 200 μm [2]; 2 201 –2 700 μm [3]; longer than 2 700 μm [4]
4	Length breadth ratio **F**	less than 2.8 [1]; 2.8 –3.4 [2]; 3.5 –4[3]; more than 4[4]
5	Length breadth ratio **M**	less than 3 [1]; 3 –3.75 [2]; 3.76 –4.5 [3]; more than 4.5 [4]
6	Wing index (α/β)	less than 1 [1]; approximate to 1 [2]; more than 1 [3]
7	δ	negative value [1]; positive value [2]
8	π	negative value [1]; positive value [2]
9	Length of antennal segment Ⅲ(A3)	shorter than 250 μm [0]; 250 –350 μm [1]; 351 –500 μm [2]; longer than 500 μm [3]
10	A3/A4 +5	less than 1 [1]; approximate to 1 [2]; more than 1 [3]
11	Length ratio of A3 and labium (A3/L) **F**	less than 1.5 [1]; 1.5 –2 [2]; more than2 [3]
12	Length ratio of A3 and labium (A3/L) **M**	less than 1.5 [1]; 1.5 –2 [2]; more than2 [3]
13	A4 –16 have uniform length	yes [0]; no [1]
14	Number of ascoid on A3 **F**	0 [0]; 1 [1]; 2 [2]
15	Number of ascoid on A3 **M**	0 [0]; 2 [1]
16	Ascoid formula **F**	1/3 –15 [1]; 2/3 –15 [2]; 2/3 –8,1/9 –15 [3]; 2/3 –14,1/15 [4]; 2/3 –12,1/13 –15 [5]; 2/3 –7,1/8 –15 [6]; 1/4 –15 [7]
17	Ascoid formula **M**	2/3 –15 [1]; 2/3 –9,1/10 –15 [2]; 1/4 –15 [3]
18	Longest palpal segment	3 [1]; 5 [2]
19	Papillary formula	12 345 [1]; 14 235 [2]; 12 435 [3]; 14 253 [4]; 14 325 [5]
20	Location of Newsead's sensilla	the basal third of segment 3 [1]; the middle of segment 3 [2]; both segments 2 and 3 [3]
21	Shape of pigment patch **F**	absent [0]; triangle [1]; mushroom shape [2]; rectangle [3]; horseshoe [4]; toadstool with a branched apex [5]; toadstool with an unbranched apex [6]
22	Shape pigment patch **M**	absent [0]; triangle [1]; mushroom shape [2]; rectangle [3]; horseshoe [4]
23	Cibarium armature **F**	absent or degenerate [1]; well developed [2]
24	Cibarium armature **M**	absent or degenerate [1]; well developed [2]
25	Structure of cibarium armature **F**	absent [0]; nonuniform triangle teeth [1]; uniform triangle teeth [2]; long point teeth [3]
26	Structure of cibarium armature **M**	absent [0]; nonuniform triangle teeth [1]; uniform triangle teeth [2]
27	Frontal teeth **F**	absent [0]; reach all transverse diameter [1]; do not reach all transverse diameter [2]
28	Frontal teeth **M**	absent [0]; reach all transverse diameter [1]; do not reach all transverse diameter [2]
29	Number of the frontal teeth rows **F**	0 [0]; 1 [1]; 2 [2]; 3 [3]; 4 [4]
30	Hind teeth **F**	0[0]; 1 –20 [1]; 21 –30 [2]; 31 –50 [3]; more than 50 [4]
31	Hind teeth **M**	0[0]; 1 –10 [1]; 11 –20 [2]; 21 –30 [3]; more than 30 [4]
32	Arrangement of teeth **F**	regular transverse line [1]; irregular fine teeth [2]; irregular transverse line [3]
33	Arrangement of teeth **M**	regular transverse line [1]; irregular fine teeth [2]; irregular transverse line [3]
34	Posterior pharynx protrudes	no [1]; yes [2]
35	Structure of pharyngeal armature **F**	transverse ridges [1]; pointed teeth and transverse ridges [2]; triangle teeth and transverse ridges [3]; scales [4]; long thin teeth [5]
36	Structure of pharyngeal armature **M**	transverse ridges [1]; pointed teeth and transverse ridges [2]; triangle teeth and transverse ridges [3]; scales [4]; pointed teeth and scales [5]; long thin teeth [6]
37	Pharyngeal armature **F**	degenerated [1]; developed [2]; well developed [3]
38	Pharyngeal armature **M**	degenerated [1]; developed [2]; well developed [3]
39	Abdominal hairs **F**	erect [1]; recumbent [2]; intermediate [3]
40	Abdominal hairs **M**	erect [1]; recumbent [2]; intermediate [3]
41	Shape of spermatheca	spheroid [1]; ellipsoid [2]; fusiform [3]; tubiform[4]; stacked dishes[5]
42	Capsule wall of spermatheca	smooth or covered by pinstripe [1]; covered by stripe or winkle [2]; covered by spines or other ornament [3]
43	Spermatheca	not sectionalize [1]; incomplete sectionalize [2]; complete sectionalize [3]
44	Number of spermatheca segments	0 [0]; 1 –10 [1]; more than 10 [2]
45	End of spermatheca	separate complete [1]; enlarged[2]; no specialty [3]
46	Trumpet-shaped membrane	absent [0]; present [1]

Table 2 continued

	Character	Character states and their values
47	Head of spermatheca	small [1]; big [2]
48	Neck of spermatheca	absent [0]; short [1]; long [2]
49	Spermatheca duct	thin and obvious [1]; enlarged, unclear demarcation with spermatheca [2]
50	Length of spermatheca duct	shorter than twice of spermatheca [1]; medium [2]; longer than fifth of spermatheca [3]
51	Common duct	absent [0]; short [1]; long [2]
52	Paramere	simple [1]; bilobed [2]; trilobed [3]; with an elliptical end surface [4]; claw-like [5]
53	Node on aedeagus	absent [0]; present [1]
54	Apex of aedeagus	Thin [1]; thick and blunt [2]
55	Shape of aedeagus	digitiform [1]; conical [2]; hammer-shaped [3]; slice-shaped [4]
56	Style	ovoid [1]; cylindrical [2]; crescent [3]
57	Process and brush of long hairs	absent [0]; present [1]
58	Number of hairs on coxite	0 – 20 [1]; 21 – 50 [2]; more than 50 [3]
59	Location of hairs on coxite	basal part [1]; dispersedly [2]; distal part [3]; basal and distal parts [4]
60	Number of spines	3[1]; 4[2]; 5[3]
61	Arrangement of spines	2 terminal, 3 middle [1]; 2 terminal, 2 subterminal [2]; 1 terminal, 1 subterminal, 2 middle [3]; 1 terminal, 2 subterminal, 1 middle [4]; 3 terminal,1 subterminal [5]; 1 terminal, 1 subterminal, 1 middle [6]; all terminal [7]
62	Accessory spine	absent [0]; located between spines [1]; located proximal of spines [2]
63	Pompetta sidebar	absent [0]; present [1]
64	Length ratio of coxite and surstyle (C/Sur)	less than 1 [1]; approximate to 1 [2]; more than 1 [3]
65	Length ratio of coxit and style (C/S)	less than 1 [1]; 1 – 1.5 [2]; 1.6 – 2 [3]; more than 2 [4]
66	Length ratio of coxit and aedeagus (C/Aed)	less than 2.5 [1]; more than 2.5[2]
67	Length ratio of paramere and aedeagus (P/Aed)	less than 1.5 [1]; 1.5 – 2.5 [2]; more than 2.5 [3]
68	Length ratio of genital filament and genital pump (GF/GP)	less than 3 [1]; 3 – 5 [2]; more than 5 [3]

F: Female; **M**: Male.

2.3 Cluster analysis

The matrix composed of taxa and characters (listed in Table 3.) was analyzed with SPSS 15.0 software using hierarchical cluster. Cluster analysis was made with cases as the object, between-groups linkage as cluster method and Chi-square measure as measure method. Values were not transformed. The result of hierarchical classification was exported with a dendrogram.

3 RESULTS

The result of cluster analysis shows that the Chinese Phlebotominae are divided into 5 groups where λ equals to 19. It is consistent with classic taxonomy in the genus classifications of Phlebotominae. The first axis separates genus *Idiophlebotomus*, genus *Phlebotomus* with genus *Chinius*, genus *Grassomyia* and genus *Sergentomyia*. The second axis separates genus *Chinius* with genus *Grassomyia* and genus *Sergentomyia*, and disparts genus *Idiophlebotomus* with genus *Phlebotomus* at the same time. Genus *Grassomyia* and genus *Sergentomyia* are disjoined by the third axis. Genus *Phlebotomus* falls into 5 groups where λ equals to 9, which agrees with subgenus classifications of genus *Phlebotomus* in classic taxonomy. Subgenus *Adlerius* unites with subgenus *Larroussius* firstly, then with subgenus *Euphlebotomus*, and later with subgenus *Anaphlebotomus*, at last they unite with subgenus *Paraphlebotomus*. However there are some differences in the subgenus classifications of genus *Sergentomyia* between cluster analysis and classic taxonomy. Genus *Sergentomyia* is separated into 8 groups where λ equals to 11.5. Two of them are respectively identical with subgenus *Sergentomyia* and *nicnic* group in classic taxonomy. *S. tangi* and *S. quanzhouensis* unite with subgenus *Parrotomyia* while the latter belongs to subgenus *Neophlebotomus* in Leng's systematics. *S. yini* (divided in subgenus *Parrotomyia* by Leng) is separated into subgenus *Neophlebotomus*. *S. iyengari*, *S. malayensis*, *S. nankingensis*, *S. lanzhouensis* and *S. turfanensis* aggregate as a group. The first three species of them are separated into subgenus *Neophlebotomus* in Leng's systematics. *S. koloshanensis* (separated into subgenus *Neophlebotomus* by Leng), *S. fanglianensis* and *S. yunnanensis* act as single groups, respectively. They join *nicnic* group first, then aggregate with the group composed of other species in genus *Sergentomyia*. The two groups enjoy a far phylogenetic relationship.

Table 3 The matrix composed by taxa and characters

Species	Characters																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
C. junlianensis	0	2	2	2	2	1	1	2	0	1	2	2	0	1	2	2	4	2	3	2	4	4	2
G. indica	1	1	1	3	4	1	2	2	0	1	1	1	1	0	0	3	7	2	1	3	5	2	2
I. longiforceps	1	3	2	2	1	3	2	2	3	3	3	3	1	1	2	1	2	1	4	1	0	0	2
P. chinensis	1	3	2	2	2	3	2	2	2	3	2	2	1	1	2	1	2	2	2	2	0	0	1
P. fengi	1	4	3	2	2	3	2	1	2	3	1	2	1	1	2	1	3	2	2	2	0	0	1
P. longiductus	1	3	3	2	2	3	2	2	2	3	1	2	1	1	2	1	6	2	2	2	0	0	1
P. sichuanensis	1	4	4	2	3	3	2	2	3	3	1	2	1	1	2	1	3	2	5	2	0	0	1
P. stantoni	1	2	2	2	2	3	2	2	1	3	1	2	1	1	2	1	2	2	2	2	0	0	1
P. kiangsuensis	1	2	2	2	2	3	2	2	1	3	1	2	1	1	2	1	2	2	2	2	0	0	1
P. tumenensis	1	3	2	2	2	3	2	1	2	3	1	2	1	1	2	1	2	2	2	2	0	0	1
P. yunshengensis	1	3	2	2	2	3	2	1	3	3	1	2	1	1	2	1	5	2	2	2	0	0	1
P. lengi	1	2	2	2	2	3	2	2	1	3	1	2	1	1	2	1	5	2	2	2	0	0	1
P. smirnovi	1	2	2	4	3	3	2	2	1	3	1	2	1	1	2	1	6	2	2	2	0	0	1
P. alexandri	1	2	2	3	3	1	2	2	0	1	1	1	1	1	2	1	2	2	2	1	0	0	1
P. mongolensis	1	3	2	3	3	2	2	2	1	3	1	1	1	1	1	1	2	2	2	1	0	0	1
P. caucasicus	1	3	2	3	3	2	2	2	1	3	1	1	1	1	2	1	2	2	2	1	0	0	1
P. andrejievi	1	2	2	3	3	2	2	2	1	3	1	1	1	1	2	1	2	2	2	1	0	0	1
S. anhuiensis	1	2	2	3	3	2	2	2	1	3	2	2	1	1	1	1	1	2	1	1	6	0	2
S. iyengari	1	2	2	4	4	2	2	2	0	3	2	2	1	1	1	1	1	2	1	1	6	0	2
S. khawi	1	2	2	3	2	2	2	2	1	3	2	2	1	1	1	1	1	2	1	1	6	0	2
S. koloshanensis	1	2	2	3	2	1	2	2	1	3	2	2	1	1	1	1	1	2	1	1	2	2	2
S. malayensis	1	2	1	4	2	2	2	2	0	3	1	2	1	1	1	1	1	2	1	1	6	0	2
S. nankingensis	1	2	2	3	3	2	2	2	1	3	2	2	1	1	1	1	1	2	1	1	6	1	2
S. quanzhouensis	1	2	2	3	2	2	2	2	1	3	2	2	1	1	1	1	1	2	1	1	5	0	2
S. squamirostris	1	2	2	3	3	2	2	2	1	3	2	2	1	1	1	1	1	2	1	1	6	0	2
S. zhengjiani	1	3	3	3	3	2	2	2	2	3	2	2	1	1	1	1	1	2	1	1	6	0	2
S. zhongi	1	2	2	3	2	1	2	2	1	3	1	2	1	1	1	1	1	2	1	1	6	2	2
S. barraudi	1	2	2	3	2	1	2	2	0	2	1	2	1	1	1	1	1	2	1	1	5	0	2
S. kwangsiensis	1	1	1	3	4	1	2	2	0	2	1	1	1	1	1	1	1	2	1	1	5	0	2
S. rudnicki	1	2	1	2	2	2	2	2	1	3	2	2	1	1	1	1	1	2	1	1	5	1	2
S. yini	1	3	3	2	2	3	2	2	2	3	2	3	1	1	1	1	1	2	1	1	5	0	2
S. sinkiangensis	1	2	2	4	4	1	2	2	0	1	1	1	1	1	1	1	1	2	1	1	6	2	2
S. sintoni	1	2	1	4	4	1	2	2	0	1	1	1	1	1	1	1	1	2	1	1	6	2	2
S. bailyi	1	1	1	2	3	1	2	2	0	2	1	2	1	1	1	1	1	2	1	1	0	0	2
S. campester	1	2	1	3	3	2	2	2	0	2	1	2	1	1	1	1	1	2	1	1	3	3	2
S. fanglianensis	1	3	3	1	1	3	2	2	2	3	2	2	1	1	1	1	1	2	1	1	0	0	2
S. yunnanensis	1	2	2	3	3	1	2	2	2	1	3	3	1	1	1	1	1	2	3	1	1	1	2
S. turfanensis	1	2	2	4	4	1	2	2	0	3	2	2	1	1	1	1	1	2	3	1	5	0	2
S. lanzhouensis	1	2	2	4	4	1	2	2	0	3	2	2	1	1	1	1	1	2	1	1	5	1	2
S. tangi	1	2	2	4	4	1	2	2	0	3	2	2	1	1	1	1	1	2	3	1	6	0	2

Table 3 continued

Species	Characters																						
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
C. junlianensis	1	1	1	0	0	0	3	3	2	2	1	2	2	2	2	1	1	5	2	2	1	3	0
G. indica	2	3	1	1	0	1	3	2	1	1	2	5	6	3	2	3	3	1	3	0	0	3	0
I. longiforceps	2	2	2	0	0	0	4	4	3	3	1	4	4	3	3	1	1	4	1	0	0	3	0
P. chinensis	1	0	0	0	0	0	0	0	2	2	1	3	3	2	2	1	1	3	2	1	2	3	0
P. fengi	1	0	0	0	0	0	0	0	2	2	1	3	3	2	2	1	1	3	2	1	2	3	0
P. longiductus	1	0	0	0	0	0	0	0	2	2	1	3	3	2	2	1	1	3	2	1	2	3	0
P. sichuanensis	1	0	0	0	0	0	0	0	2	2	1	3	3	2	2	1	1	3	2	1	2	3	0
P. stantoni	1	0	0	0	0	0	0	0	2	2	1	3	1	2	1	1	1	4	2	2	2	3	0
P. kiangsuensis	1	0	0	0	0	0	0	0	2	2	1	2	1	2	1	1	1	4	2	2	1	1	0
P. tumenensis	1	0	0	0	0	0	0	0	2	2	1	2	2	2	2	1	1	2	2	2	1	1	0
P. yunshengensis	1	0	0	0	0	0	0	0	2	2	1	2	2	2	2	1	1	2	2	2	1	1	0
P. lengi	1	0	0	0	0	0	0	0	2	2	1	2	2	2	2	1	1	3	2	2	2	1	0
P. smirnovi	1	0	0	0	0	0	0	0	2	2	1	2	2	2	2	1	1	3	2	2	2	3	0
P. alexandri	1	0	0	0	0	0	0	0	2	2	1	4	4	3	3	1	1	5	2	2	1	2	0
P. mongolensis	1	0	0	0	0	0	0	0	2	2	1	4	4	3	3	1	1	5	2	2	1	2	0
P. caucasicus	1	0	0	0	0	0	0	0	2	2	1	4	4	3	3	1	1	5	2	2	1	2	0
P. andrejievi	1	0	0	0	0	0	0	0	2	2	1	4	4	3	3	1	1	5	2	2	1	2	0
S. anhuiensis	2	3	2	1	1	1	3	3	1	1	2	2	5	3	3	2	2	4	1	0	0	3	0
S. iyengari	2	2	2	2	1	2	1	2	1	1	2	1	1	1	1	3	2	4	1	0	0	3	1
S. khawi	2	2	2	1	1	3	2	3	1	1	2	4	4	3	3	2	2	4	1	0	0	3	0
S. koloshanensis	2	1	1	0	0	0	1	1	3	1	2	1	1	1	1	2	2	4	1	0	0	3	1
S. malayensis	2	2	2	1	2	3	1	1	1	1	2	1	1	1	1	2	2	4	1	0	0	3	1
S. nankingensis	2	2	2	1	0	1	1	1	1	1	2	1	1	1	1	2	2	4	1	0	0	3	0
S. quanzhouensis	2	2	2	1	2	1	1	2	1	1	2	4	4	3	3	2	2	4	1	0	0	3	1
S. squamirostris	2	3	2	1	0	1	4	3	1	1	2	5	5	3	3	2	2	4	1	0	0	3	0
S. zhengjiani	2	2	2	1	2	4	2	2	1	1	2	5	5	3	3	2	2	4	1	0	0	3	0
S. zhongi	2	2	2	1	1	2	1	1	1	1	2	4	4	3	3	2	2	4	1	0	0	3	0
S. barraudi	2	3	2	1	2	1	4	2	1	1	2	5	4	3	3	2	2	2	1	0	0	3	0
S. kwangsiensis	2	3	2	1	2	1	4	2	1	1	2	5	1	3	1	2	2	2	1	0	0	3	0
S. rudnicki	2	3	2	1	1	2	4	3	1	1	2	5	6	3	3	3	2	2	1	0	0	3	0
S. yini	2	3	2	1	1	2	4	3	1	1	2	5	6	3	2	2	2	2	1	0	0	3	0
S. sinkiangensis	2	2	1	1	2	1	1	1	1	3	1	5	1	3	1	2	2	4	1	0	0	3	0
S. sintoni	2	2	1	1	2	1	1	2	1	3	1	5	1	3	1	2	2	4	1	0	0	3	0
S. bailyi	2	2	2	0	0	0	1	1	3	3	2	2	2	2	2	2	2	2	1	0	0	3	0
S. campester	2	2	2	0	0	0	1	1	3	3	2	2	2	2	2	2	2	4	1	0	0	3	0
S. fanglianensis	2	2	2	0	0	0	2	3	3	3	2	1	1	1	2	2	2	4	1	0	0	3	0
S. yunnanensis	2	1	1	0	0	0	1	1	3	3	1	1	1	1	2	2	2	4	1	2	2	3	0
S. turfanensis	2	2	2	1	0	1	1	1	1	1	2	1	1	1	1	2	2	2	1	0	0	3	0
S. lanzhouensis	2	2	2	1	1	1	2	2	1	1	2	1	1	1	1	2	2	4	1	0	0	3	0
S. tangi	2	2	2	2	2	1	1	2	1	1	2	4	4	3	3	2	2	2	1	0	0	3	0

Table 3 continued

Species	Characters																					
	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
C. junlianensis	1	0	1	2	3	1	0	2	3	3	0	2	1	2	4	0	0	1	1	1	3	3
G. indica	1	0	1	2	1	1	0	1	2	2	0	1	2	2	5	2	1	3	4	1	2	2
I. longiforceps	1	0	1	1	1	5	0	1	2	2	0	2	3	1	6	0	0	1	1	1	3	1
P. chinensis	1	2	1	2	2	2	1	1	1	2	0	3	1	3	1	0	0	1	3	1	2	3
P. fengi	1	2	1	3	1	2	1	1	1	2	0	2	1	3	1	0	0	1	3	1	1	3
P. longiductus	1	2	1	3	1	2	1	1	1	2	0	3	1	3	1	0	0	1	3	1	1	3
P. sichuanensis	1	2	1	3	1	2	1	1	1	2	0	2	1	3	1	0	0	1	3	1	2	3
P. stantoni	2	1	1	1	3	3	0	1	2	1	0	2	1	2	3	0	0	2	4	1	1	1
P. kiangsuensis	1	0	1	2	1	3	0	1	2	1	0	1	2	3	1	0	0	1	3	2	3	1
P. tumenensis	1	1	1	2	1	3	0	1	2	1	0	1	2	3	1	0	0	1	3	2	3	1
P. yunshengensis	1	1	1	3	1	3	0	1	2	1	0	3	1	3	1	0	0	1	3	2	2	1
P. lengi	1	2	1	2	1	3	0	1	2	1	0	1	2	3	1	0	0	1	3	1	2	2
P. smirnovi	2	2	1	2	2	1	0	1	1	2	0	1	2	3	1	0	0	1	3	1	1	2
P. alexandri	1	0	1	2	1	4	0	2	2	1	1	1	2	2	3	0	0	1	3	2	2	1
P. mongolensis	1	0	1	3	1	4	0	2	2	1	1	1	2	2	3	0	0	1	2	2	2	1
P. caucasicus	1	0	1	2	1	4	0	2	2	1	1	1	2	2	3	0	0	1	3	2	3	1
P. andrejievi	1	0	1	2	1	4	0	2	2	1	1	1	2	2	3	0	0	1	3	2	2	1
S. anhuiensis	1	0	1	1	2	1	0	1	2	2	0	2	1	2	2	2	0	3	4	2	2	2
S. iyengari	1	0	1	1	2	1	0	1	2	2	0	1	2	2	7	2	0	3	4	2	3	2
S. khawi	1	0	1	1	2	1	0	1	2	2	0	1	2	2	2	2	0	3	4	2	2	2
S. koloshanensis	1	0	2	1	2	1	0	1	2	2	0	1	1	2	2	2	0	3	3	2	2	2
S. malayensis	1	0	1	1	2	1	0	1	2	2	0	1	2	2	7	2	0	3	4	2	3	2
S. nankingensis	1	0	1	1	2	1	0	1	2	2	0	1	2	2	2	2	0	3	3	2	2	1
S. quanzhouensis	1	0	1	1	2	1	0	1	2	2	0	3	1	2	7	2	0	3	3	1	2	2
S. squamirostris	1	0	1	1	2	1	0	1	2	2	0	3	1	2	2	2	0	3	3	2	2	2
S. zhengjiani	1	0	1	1	3	1	0	1	2	2	0	2	1	2	2	2	0	3	4	1	1	2
S. zhongi	1	0	1	1	2	1	0	1	2	2	0	2	1	2	2	2	0	3	4	2	2	2
S. barraudi	1	0	1	2	2	1	0	1	2	2	0	1	2	2	7	2	0	2	4	1	2	2
S. kwangsiensis	1	0	1	1	2	1	0	1	2	2	0	1	2	2	7	2	0	3	4	1	2	2
S. rudnicki	1	0	1	1	2	1	0	1	2	2	0	1	2	2	7	2	0	3	4	1	2	2
S. yini	1	0	1	1	2	1	0	1	2	2	0	3	1	2	2	1	0	3	4	2	2	2
S. sinkiangensis	1	0	2	1	2	1	0	2	2	2	0	1	2	2	7	2	0	3	4	2	2	2
S. sintoni	1	0	2	1	2	1	0	2	2	2	0	1	2	2	7	2	0	3	4	2	2	2
S. bailyi	1	0	1	1	1	1	0	1	2	2	0	1	2	2	7	2	0	3	4	2	2	2
S. campester	1	0	1	1	1	1	0	1	2	2	0	1	2	2	7	2	0	3	4	2	2	2
S. fanglianensis	1	0	1	1	2	1	0	1	2	2	0	2	1	2	2	2	0	3	3	2	2	2
S. yunnanensis	1	0	1	1	2	1	0	1	4	2	0	3	4	2	2	1	0	3	3	2	1	2
S. turfanensis	1	0	1	1	2	1	0	1	2	2	0	1	2	2	7	2	0	3	4	2	2	2
S. lanzhouensis	1	0	1	1	2	1	0	1	2	2	0	1	2	2	7	2	0	1	4	2	2	2
S. tangi	1	0	1	1	2	1	0	1	2	2	0	1	2	2	7	2	0	1	4	2	2	2

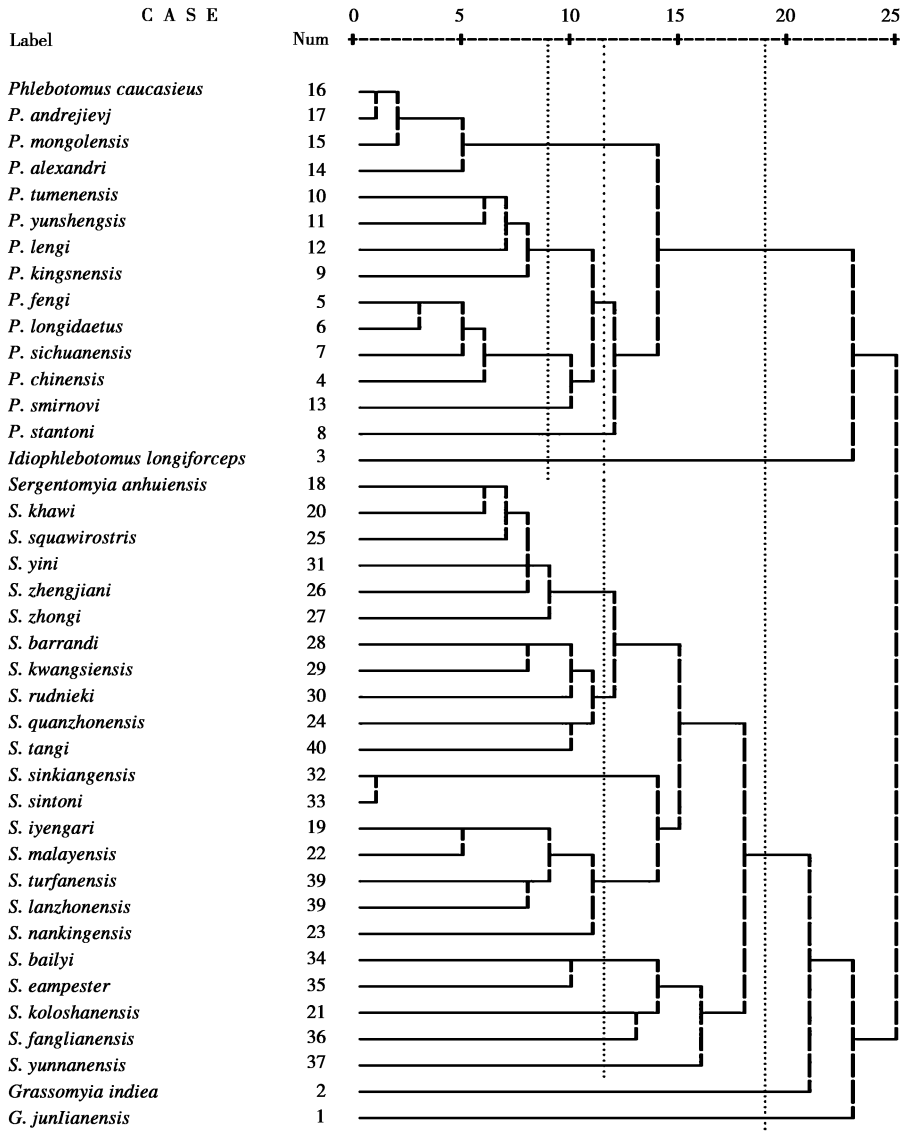

Fig. 1 The dendrogram of Chinese species of Phlebotominae constructed using between-groups linkage

4 DISCUSSION

There are about 700 species of Phlebotominae reported in the world, which are divided equally between the Old World and the New World. The systematics of the Phlebotominae in the Old World and the New World is independent for the very different morphology. This paper just deals with the systematics in the Old World. Classification of the Phlebotominae began in 1911 and some aspects are still debatable. Theodor (1948) used some morphological characters as index to identify genus, which include male genital apparatus, spermatheca, pharynx armature, cibarium armature, pigment patch, abdominal hairs and sexual dimorphism of oral cavity development, *etc* ., and established a classification system of Phlebotominae called Theodor's systematics that was recognized by the academic field though there were some different points. Artemiev (1984) of the former Soviet Union advised to establish 24 supra-specific groups, subgenera and genera that induced lots of single-species-genus, but was not accepted.

According to Lewis' opinion, Theodor's subgenera of *Sergentomyia* have not been universally adopted, and even Theodor (1958) evidently regarded *Neophlebotomus* as not very well defined. It was treated with some reservations, and included two exceptional species when dealing with the Palaearctic Region only. However, the subgenera are still used by Lewis (1978) for avoiding an unduly large assemblage of ungrouped species, and facilitating faunal comparison with other regions. Some Oriental species of *Sergentomyia* are easily placed in the subgenera *Parrotomyia* and *Neophlebotomus*, but others less easily. Many, however, outside the distinct *Sergentomyia* and *Sintonius*, form a miscellaneous category of species, some of which may be loosely connected with the subgenera.

Lewis *et al.* (1977) found a stable classification of the phlebotomine sandflies following Theodor's systematics. Phlebotominae was divided into genus *Phlebotomus* (including subgenus *Phlebotomus*, *Paraphlebotomus*, *Synphlebotomus*, *Larroussius*, ungrouped, subgenus *Idiophlebotomus* and *Anaphlebotomus*) and genus *Sergentomyia* (including *musai*-group, subgenus *Sergentomyia*, *Parrotomyia*, *Grassomyia*, *Neophlebotomus*, *nicnic*-group, ungrouped and subgenus *Sintonius*). At present there are 6 genera recognized in the world including *Phlebotomus* Rondani & Berte, *Sergentomyia* Franca & Parrot and *Chinius* Leng in the Old World, and *Lutzomyia* Franca, *Brumptomyia* Franca & Parrot, *Warileya* Hertig in the New World. Artemiev and Neronov (1984) as well as Leng (1997) upgraded *Idiophlebotomus* Quate & Fairchild and *Grassomyia* Theodor as genus. The systematics of Leng (1997) about Chinese Phlebotominae is following Lewis' classification system and Theodor's systematics.

The result of cluster analysis is consistent with Leng's systematics on the whole, and it is completely identical at the genus level and the subgenus level of genus *Phlebotomus*. Rispail and Léger (1998) did numerical taxonomy of the Old World Phlebotominae including some species of Chinese Phlebotominae. The results obtained by using different characters and methods coincide with each other, which reveals the stabilization of the classification and the uniform morphology in subgenera of genus *Phlebotomus*. However, the result of cluster analysis differs from Leng's systematics in genus *Sergentomyia* in a way. The taxonomic positions of subgenus *Sergentomyia* and *nicnic* group are identical, respectively, both in cluster analysis and classic taxonomy. The difference lies in the classification of subgenus *Neophlebotomus* and *Parrotomyia*, which reveals that the uncertain phenomenon of some species in Leng's systematics inherited Theodor's systematics. *S. tangi* and *S. quanzhouensis* unite with subgenus *Parrotomyia* while the latter was separated into subgenus *Neophlebotomus* by Leng. *S. yini* (divided in subgenus *Parrotomyia* by Leng) is separated into subgenus *Neophlebotomus*. *S. iyengari*, *S. malayensis* and *S. nankingensis* (all belong to subgenus *Neophlebotomus* in classic taxonomy) aggregate with *S. lanzhouensis* and *S. turfanensis* as a group at the subgenus level. Their morphology characters were compared with subgenus *Sintonius* which is absent in China. The result shows that they do not belong to subgenus *Sintonius* which is characterized with segmented spermatheca (Lewis, 1977). Therefore, it is suggested that they should hold a new multispecies subgenus or group at the subgenus level. *S. koloshanensis* (separated into subgenus *Neophlebotomus* by Leng). *S. fanglianensis* and *S. yunnanensis* act as single groups, respectively for their particular morphology. Taking the specialties into account, *S. fanglianensis* and *S. yunnanensis* were listed in ungrouped by Leng. *S. koloshanensis*, *S. fanglianensis*, *S. yunnanensis* and *nicnic* group may occupy a new genus for their far phylogenetic relationships with others of genus *Sergentomyia*. This point is agreed by Leng to some extent, who thought that *S. fanglianensis* and *nicnic* group probably should be treated as a new subgenus or genus.

In this situation, numerical taxonomy would be more comprehensive, objective and practical. In view of the species and morphological characters used following the classic classifications, as well as numerical taxonomy serving as an exploring method, more researches are yet required on the revelations that numerical taxonomy gives us.

The classic classification based on the morphology of adult sandflies is still widely accepted. The present study confirms the soundness of classic classifications, while refining the taxonomic levels. We hope that it would be helpful to other research in classification at molecular and genetic levels.

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中国白蛉亚科数值分类研究
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摘要:【目的】对中国白蛉亚科(Phlebotominae)昆虫进行数值分类研究, 探索其在系统发育过程中的亲缘关系。【方法】选取中国白蛉 40 个蛉种作为分类单元以及白蛉的 68 项形态特征为分类指标, 进行系统聚类分析。【结果】聚类分析结果与传统属级及白蛉属 *Phlebotomus* 的亚属级分类一致。司蛉属 *Sergentomyia* 的亚属分类与传统分类有一些区别, 聚类分析将司蛉属分为 8 类, 其中尼克组 *nicnic* group 与司蛉亚属 subgenus *Sergentomyia* 和传统分类一致; 泉州司蛉 *S. quanzhouensis* 和唐氏司蛉 *S. tangi* 聚入帕蛉亚属 subgenus *Parrotomyia*; 尹氏司蛉 *S. yini* 聚入新蛉亚属 subgenus *Neophlebotomus*, 应氏司蛉 *S. iyengari*、马来司蛉 *S. malayensis*、吐鲁番司蛉 *S. turfanensis*、兰州司蛉 *S. lanzhouensis* 和南京司蛉 *S. nankingensis* 聚为一类; 歌乐山司蛉 *S. koloshanensis*、方亮司蛉 *S. fanglianensis* 和云南司蛉 *S. yunnanensis* 与尼克组 *nicnic* group 聚成一类, 此类群和此属中其他蛉种系统发育关系距离远。【结论】中国白蛉亚科昆虫的聚类分析结果与传统分类基本一致, 验证了传统分类系统的可靠性, 并揭示了传统分类中一些蛉种不确定分类问题。司蛉属聚类分析结果与传统分类有差别, 提示有个别蛉种需重新考虑其分类地位。

关键词: 双翅目; 毛蠓科; 白蛉亚科; 白蛉; 形态特征; 数值分类; 聚类分析; 中国
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